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Escape from the checkpoint: Nek2A binds a unique conformation of the APC/C-MCC complex

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Cell division depends on the timely degradation of numerous proteins by the anaphase-promoting complex/cyclosome (APC/C). The APC/C is a large E3 ubiquitin ligase that in complex with Cdc20 recognises degrons in its substrates. The ability of APC/C-Cdc20 to bind degrons is prevented by the binding of the mitotic checkpoint complex (MCC) which constitutes the "wait anaphase" signal. Curiously, the mitotic kinase Nek2A is insensitive to the presence of the MCC. How Nek2A avoids MCC inhibition has been unclear but now work from Alfieri and colleagues published in this issue of EMBO reports provides an explanation [1]. It shows that Nek2A is able to bind a specific open conformation of the APC/C-MCC complex that allows Nek2A ubiquitination. A dimer of Nek2A binds two distinct binding pockets on the APC/C through C-terminal MR motifs and thus independently of degrons. One of the MR binding pockets is only available for interaction in the open form of APC/C-MCC explaining Nek2A selectivity for this conformation. Whether other substrates bind the APC/C directly without using canonical degrons will be important to determine.

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See also: **C Alfieri *et al*** (June 2020)

During cell division, the duplicated genetic material has to be equally segregated to the two new daughter cells. Key to avoiding errors in chromosome segregation is the degradation of specific proteins at the right time. The APC/C is the E3 ubiquitin ligase responsible for targeting mitotic regulators for proteasomal degradation during mitosis. It consists of 20 subunits with a total molecular mass of 1.2 MDa yet is fully dependent on the protein Cdc20 for activity during mitosis [2]. The role of Cdc20 is twofold namely to help in recruitment of substrates and in activation of the APC/C. APC/C-Cdc20 binds substrates by recognising a number of short degradation motifs (degrons) including D-box, KEN box and ABBA motifs [3]. These motifs bind to specific pockets on Cdc20, and a single substrate can contain multiple motifs. Activation of the APC/C by Cdc20 is achieved by the binding of a C-box motif in Cdc20 to the APC8 subunit hereby inducing a conformational change that leads to APC/C activation. In addition, Cdc20 binds to a pocket on the APC3 subunit through an extreme C-terminal isoleucine–arginine (IR) motif.

Important mitotic substrates of the APC/C are cyclin B1 and securin. Cyclin B1 is the activating subunit of cdk1, the major mitotic kinase, while securin is an inhibitor of separase, the enzyme that cleaves cohesin to allow sister chromatid separation. The degradation of cyclin B1 and securin only starts when all chromosomes have aligned at the metaphase plate, and the spindle assembly checkpoint (SAC) has been satisfied. The SAC prevents APC/C-Cdc20 activity prior to metaphase by generating the mitotic checkpoint complex (MCC), a diffusible and potent inhibitor of the APC/C-Cdc20 complex. The MCC consists of the checkpoint proteins Mad2 and BubR1-Bub3 bound to Cdc20. This complex specifically binds APC/C-Cdc20 such that the final assembled complex contains two copies of Cdc20, one from the MCC complex ($Cdc20^{MCC}$) and one from the APC/C complex ($Cdc20^{APC/C}$). The IR motif of $Cdc20^{MCC}$ binds to a pocket on the APC8 subunit rather than to APC3. BubR1 acts as a pseudosubstrate and blocks the degron binding pockets of both Cdc20 molecules hereby potently inhibiting APC/C activity [4]. Structural analysis of the APC/C-MCC complex has revealed that it exists in two major conformations referred to as open and closed. In the open conformation, the binding of the E2 enzyme UbcH10, that brings activated ubiquitin to the APC/C, is possible while in the closed it is not. The closed form of the APC/C-MCC thus represents a more potently inhibited state (Fig 1).

Curiously, a number of APC/C-Cdc20 substrates have been identified that escape inhibition by the SAC and these substrates are degraded as soon as cells enter mitosis. The early degradation of two of these substrates, cyclin A2 and the kinase Nek2A, is required for mitotic fidelity. A number of studies have clarified how cyclin A2 uses a unique combination of degrons as well as the Cks protein to bind the open conformation of APC/C-MCC for efficient degradation [5,6]. However, Nek2A is different from cyclin A2 in that its degradation is not strongly dependent on canonical degrons. Rather Nek2A has an extreme C-terminal methionine–arginine (MR) motif required for binding and degradation (Fig 1, box) [7–9]. This MR motif is reminiscent of the IR motif found in Cdc20 and the G1 APC/C co-activator Cdh1. Furthermore, previous work had shown that dimerisation of Nek2A through its leucine zipper is

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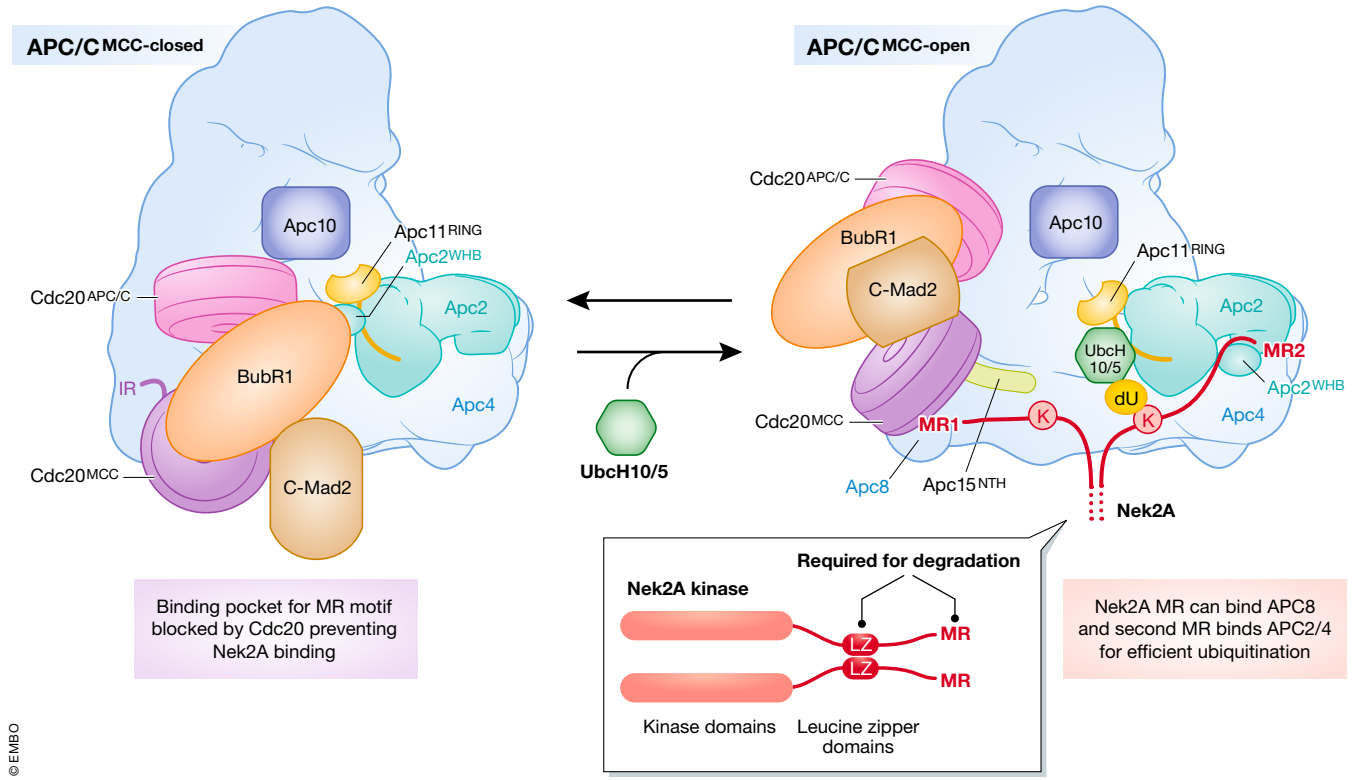

Figure 1. Nek2A binds the APC/C-MCC complex in a unique conformation.

Nek2A is a mitotic kinase that is degraded by the APC/C despite this being inhibited by the spindle assembly checkpoint. The APC/C is inhibited by the checkpoint generated MCC, and two specific conformations of this complex exist referred to as closed and open. In the closed form, the APC/C-MCC is more strongly inhibited and the IR motif of $Cdc20^{MCC}$ binds APC8 preventing Nek2A binding. In the open form of APC/C-MCC, this $Cdc20^{MCC}$-APC8 interaction is weaker allowing Nek2A to bind APC8 through one of its MR motifs. The second MR motif of the Nek2A dimer binds to an APC2/4 interface hereby stabilising the interaction. This unique binding mechanism possibly positions Nek2A lysine residues for efficient ubiquitination by UbcH10 or UbcH5 (modified from ref. [1])

important for its degradation during an active SAC [10]. However, whether Nek2A competes with the MCC for APC/C binding or uses a unique mechanism to be degraded by the APC/C-MCC complex has been unclear. In this issue of *EMBO Reports*, Alfieri and colleagues provide a detailed structural and functional characterisation of the APC/C-Nek2A complex to provide answers.

The researchers generate a 3D reconstruction of the APC/C-Nek2A complex at 3.9 Å resolution using cryo-EM and find density for an MR motif in the APC8 subunit. In the APC/C-MCC structure, this MR binding pocket on APC8 is occupied by the IR motif of $Cdc20^{MCC}$. Improving their previous cryo-EM reconstructions of APC/C-MCC in the closed and open forms, it is evident that the density for the IR motif of $Cdc20^{MCC}$ is less clearly defined in the open form suggesting a less tight interaction. Based on these observations, the authors hypothesise that the Nek2A MR motif competes with the $Cdc20^{MCC}$ IR motif for binding to APC8. Since the IR motif of $Cdc20^{MCC}$ is less tightly bound in the open form of the APC/C-MCC, it will be more accessible to the Nek2A MR motif. To test this, they deplete the APC15 subunit, a non-physiological condition that stabilises the closed form of the APC/C-MCC complex. This perturbation results in reduced Nek2A binding. In contrast, using a BubR1 mutant that favours the open form or mutating the $Cdc20^{MCC}$ IR motif allows stronger binding of Nek2A. Collectively, these results support a model where the MR motif of Nek2A binds APC8, an interaction that is only possible in the open form of APC/C-MCC (Fig 1, right). Spurred by the observation that Nek2A has to be able to dimerise for degradation, the authors inspect the APC/C-Nek2A cryo-EM map for additional densities. This allows them to find density for a second MR motif at an interface between the APC4 and APC2 subunits. This MR binding site is unique for Nek2A and not occupied by the IR motifs of co-activators. However, while the APC2/4 MR binding pocket plays a critical role in Nek2A ubiquitination, it is not essential for Nek2A binding. One possible explanation is that the second MR motif helps to position the lysines of Nek2A in the active site to ensure efficient ubiquitination.

Another interesting aspect of the APC/C-Nek2A structure is the conformation of the "winged-helix B" (WHB domain) of APC2. APC2 is the cullin subunit of the APC/C complex that binds the E2 enzymes UbcH5 and UbcH10 through its WHB domain [2]. In APC/C-MCC closed, the APC2 WHB domain is unable to bind these E2 enzymes due to interactions with BubR1 (Fig 1, left). However, in the APC/C-Nek2A complex the E2 binding region of the WHB domain is exposed and the entire domain is in a different position compared to the APC/C-UbcH10 structure (Fig 1, right). Given the fact that Nek2A binding affects the APC2 WHB domain preventing BubR1 interactions, this further favours the open conformation of APC/C-MCC. That

Nek2A influences the WHB domain conformation raises the question as to whether this affects the E2 enzymes able to target Nek2A for degradation. The authors therefore compare the requirements for UbcH10 and UbcH5 *in vitro* and *in vivo*. These experiments reveal a striking difference between cyclin A2 and Nek2A. Nek2A can use both UbcH10 and UbcH5, while cyclin A2 needs UbcH10 for efficient ubiquitination. Although the structure of Nek2A bound to APC-MCC is not known, it can be that a unique conformation of the APC2 WHB domain combined with stable Nek2A binding provides flexibility in E2 utility.

Taken together, the results by Alfieri and colleagues provide a molecular understanding for how Nek2A can avoid MCC inhibition. Why Nek2A has evolved such a unique mechanism to avoid the SAC is unclear as many cell lines also express Nek2B, which lacks the C-terminus and is thus not a substrate of the APC/C. Possibly during development or in specific cell lines, Nek2A is not only a substrate of the APC/C but also a regulator of its activity. Clearly, Nek2A would be in a prime position to regulate the phosphorylation status of the APC/C or associated proteins either through its kinase activity or through its binding to the PP1 phosphatase. Exploring this could unravel novel interesting aspects of APC/C and Nek2A biology.

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